Immunofluorescence Staining

1. Culture cells of interest (BCLA+CAEV) overnight in 5 mL of liquid LB with appropriate antibiotic. Grow a control culture of plain *E. coli* in the same way.
2. Transfer to labelled 1.5 mL eppendorf tubes and centrifuge at ~13,000 rpm to pellet. Discard LB.
3. Wash by resuspending in PBS and centrifuging again. Discard PBS.
4. Resuspend cells in 500 uL 4% paraformaldehyde and leave rotating for 20 minutes at room temperature.
5. Wash 3x in PBS. Discard PBS.
6. Resuspend in 600 uL 5% BSA in PBS (1x) and leave rotating for 1 hour at room temperature.
7. Distribute 200 uL of BSA resuspension into 3 labelled 1.5 mL eppendorf tubes per culture.
8. Add 1° antibody to a concentration of 1:200 (1 uL if 200 uL in each tube). There should be three different tubes for each culture, as described in step 7: one with the antibody of interest (CAEV for us), one with the *E. coli* antibody as a control, and one with no antibody. Let rest for 1 hour.
9. Wash 3x in PBS. Discard PBS.
10. Resuspend each tube in 200 uL 5% BSA in PBS (1x) and add Alexa 568 + corresponding 2° antibody to a concentration of 1:1000 (a dilution in BSA may be necessary, 1 uL antibody to 9 uL of BSA and add 2 uL to tube). Cover tube completely in foil and leave rotating for 1 hour.
11. Wash 3x in PBS. Discard PBS.
12. Resuspend in PBS.